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IN RE APPLICATION OF: David FAIRLIE, et al

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INTERNATIONAL FILING DATE: 25 JUNE 1998

FOR: CYCLIC AGONISTS AND ANTAGONISTS OF C5a RECEPTORS AND G PROTEIN-COUPLED RECEPTORS

REQUEST FOR PRIORITY UNDER 35 U.S.C. 119
AND THE INTERNATIONAL CONVENTION

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

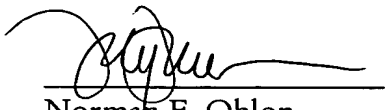
In the matter of the above-identified application for patent, notice is hereby given that the applicant claims as priority:

COUNTRY	APPLICATION NO	DAY/MONTH/YEAR
AUSTRALIA	PO 7550	25 JUNE 1997

Certified copies of the corresponding Convention application(s) were submitted to the International Bureau in PCT Application No. **PCT/AU98/00490**. Receipt of the certified copy(s) by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Norman F. Oblon
Attorney of Record
Registration No. 24,618
William E. Beaumont
Registration No. 30,996

Crystal Square Five
Fourth Floor
1755 Jefferson Davis Highway
Arlington, Virginia 22202
(703) 413-3000

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I, KIM MARSHALL, MANAGER EXAMINATION SUPPORT AND SALES, hereby certify that the annexed is a true copy of the Provisional specification in connection with Application No. PO 7550 for a patent by THE UNIVERSITY OF QUEENSLAND filed on 25 June 1997.

I further certify that the annexed specification is not, as yet, open to public inspection.

WITNESS my hand this Eighth day of July 1998

KIM MARSHALL
MANAGER EXAMINATION SUPPORT AND SALES

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Applicant(s): THE UNIVERSITY OF QUEENSLAND

Invention Title: RECEPTOR AGONIST AND ANTAGONIST

The invention is described in the following statement:

RECEPTOR AGONISTS AND ANTAGONISTS

This invention relates to antagonists of G protein-coupled receptors. In particular, the invention relates to antagonists of proteins of the complement cascade, most particularly antagonists of the plasma protein complement 5a (C5a). In preferred embodiments, the invention provides cyclic peptidic and cyclic or non-cyclic non-peptidic antagonists of C5a. The compounds of the invention are both potent and selective, and are useful in the treatment of a variety of inflammatory conditions.

BACKGROUND OF THE INVENTION

Activation of human complement, a system of plasma proteins involved in immunological defence against infection and injury, contributes significantly to the pathogenesis of numerous acute and chronic diseases. For general reviews, see Whaley (1987), and Sim (1993).

During host defence, the complement system of plasma proteins, many of which are proteases, initiates inflammatory and cellular immune responses to stimuli such as infectious organisms (bacteria, viruses, parasites), chemical or physical injury, radiation or neoplasia. Complement is activated through a complex cascade of interrelated proteolytic events that produce multiple bioactive peptides, some of which (*eg.* anaphylatoxins C3a and C5a) interact with cellular components to propagate inflammatory processes. Complement activation, either by the classical pathway, after antigen-antibody (Ag/Ab) binding, or by the antibody-independent alternate pathway, ends with a *terminal sequence* in which protein C5 is proteolytically cleaved by C5 convertase to C5a and C5b. The latter facilitates assembly of a "membrane attack complex" that punches holes in membranes of target cells (*eg.* bacteria), leading to leakage, lysis and cell death. Steps in the cascade are tightly regulated to avoid stepwise amplification of proteolysis by sequentially

formed proteases. If these regulatory mechanisms become inefficient, protracted activation of complement can result, causing enhanced inflammatory responses as in autoimmune diseases.

Although the broad features of the complement system and its activation are known, mechanistic details remain poorly understood. A principal and very potent mediator of inflammatory responses is the plasma glycoprotein C5a, which interacts with specific surface receptors (C5aR) on mast cells, neutrophils, monocytes, macrophages and vascular endothelial cells (Gerard and Gerard, 1994). C5aR is a G protein-coupled receptor with seven transmembrane helices (Gerard and Gerard, 1991). This receptor is one of the rhodopsin superfamily of GTP-linked binding proteins, but differs from rhodopsin receptors in that the receptor and G protein are linked prior to activation. C5a receptors are found on a variety of tissues and cells, including mast cells, neutrophils, monocytes, macrophages, non-myeloid cells and vascular endothelial cells (Gerard and Gerard, 1994).

G protein-coupled receptors are prevalent throughout the human body, and comprise approximately 80% of known cellular receptor types, and mediate signal transduction across the cell membrane for a very wide range of endogenous ligands. They participate in a diverse array of physiological and pathophysiological processes, including, but not limited to those associated with cardiovascular, central and peripheral nervous system, reproductive, metabolic, digestive, immunoinflammatory, and growth disorders, as well as other cell-regulatory and proliferative disorders. It is expected that agents (agonists or antagonists) which selectively modulate functions of G coupled receptors will have important therapeutic applications.

C5a is one of the most potent chemotactic agents known, and recruits neutrophils and macrophages to sites of injury, alters their morphology; induces degranulation;

increases calcium mobilisation, vascular permeability (oedema) and neutrophil adhesiveness; contracts smooth muscle; stimulates release of inflammatory mediators (including histamine, TNF-α, IL-1, IL-6, IL-8, prostaglandins, leukotrienes) and lysosomal enzymes; promotes formation of oxygen radicals; and enhances antibody production (Zuiderberg *et al*, 1980). Overexpresion or underregulation of C5a is implicated in the pathogenesis of immunoinflammatory conditions such as rheumatoid arthritis, adult respiratory distress syndrome (ARDS), systemic lupus erythematosus, tissue graft rejection, ischaemic heart disease, reperfusion injury, septic shock, psoriasis, gingivitis, atherosclerosis, Alzheimer's disease, lung injury and extracorporeal post-dialysis syndrome (Greer, 1985).

New agents which limit the pro-inflammatory actions of C5a have potential for inhibiting chronic inflammation, and its accompanying pain and tissue damage. For these reasons, molecules which prevent C5a binding to its receptors are useful for treating chronic inflammatory disorders driven by complement activation. Importantly, such compounds provide valuable new insights to mechanisms of complement-mediated immunity.

One approach to the development of antagonists of C5a is through receptor-based design, using knowledge of the three-dimensional structures of C5a, its receptor C5aR, and interactions between them.

Human C5a is a 74 amino acid peptide that is highly cationic, and is N-glycosylated with a 3 kDa carbohydrate at Asn64. The NMR solution structure of C5a has been determined, and, although it is essentially a 4-helix bundle, the C-terminal end was found to be unstructured (Zuiderberg *et al*, 1989). Indeed this conformational flexibility in the C-terminus has made structure-function studies extremely difficult to interpret.

C5a has a highly ordered N-terminal core domain (residues 1-64; $C5a_{1-64}$) consisting of a compact antiparallel 4-helix bundle (residues 4-12, 18-26, 32-39, 46-63) connected by loops (13-17, 27-31, 40-45) and further stabilised by 3 disulphide bonds (C21-Cys47, Cys22-Cys54, Cys34-Cys55). In contrast, there is no well-defined structure for the C-terminal domain (65-74, $C5a_{65-74}$) (Zuiderberg *et al*, 1989).

The structure of C5aR is unknown, although the C5a-binding subunit of human monocyte-derived C5aR has been cloned and identified as a G protein-coupled receptor with transmembrane helices (Gerard and Gerard, 1991). Interactions between C5a and C5aR have been the subject of many investigations which, in summary, suggest that C5a binds via a two-site mechanism (Figure 1) in which the N-terminal core domain of C5a is involved in receptor-recognition and binding, while the C-terminus is responsible for receptor activation. The C-terminal "effector" region alone possesses all the information necessary for signal transduction, and is thought to bind in the receptor's interhelical region (Sicilliano *et al*, 1994; deMartino *et al*, 1994)).

An N-terminal interhelical positively-charged region of C5a is responsible for receptor recognition and binding, and binds to a negatively- charged extracellular domain of C5aR (site 1), while the C-terminal "effector" region of C5a is thought to bind with the interhelical region of the receptor (site 2), and is responsible for receptor activation leading to signal transduction (Sicilliano *et al*, 1994).

Numerous short peptide derivatives of the C-terminus of C5a have been found to be agonists of C5a (Kawai *et al*, 1990; Kawai *et al*, 1991; Kawai *et al*, 1992; Kohl *et al*, 1993; Drapeau *et al*, 1993; Ember *et al*, 1992; Sanderson *et al*, 1994; Sanderson *et al*, 1995; Finch *et al*, 1997; Tempero *et al*, 1997; Konteatis *et al*, 1994; DeMartino *et al*, 1995). High molecular weight polypeptide

inhibitors, such as monoclonal antibodies, are also known (Morgan *et al*, 1992).

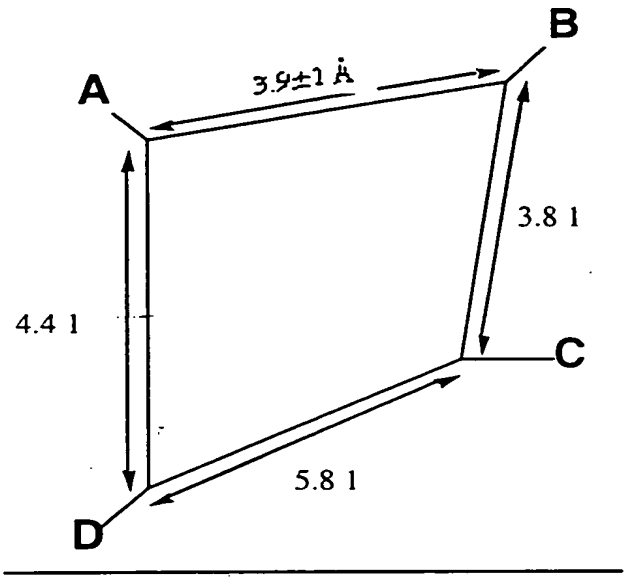
Only one small molecule, N-methylphenyl-alanine-proline-D-cyclohexylalanine-tryptophan-D-arginine (**1**, NMeF-K-P-dCha-W-r), is reported to be a full antagonist of the C5a receptor, with no agonist activity when tested on isolated cells and membranes. This hexapeptide was developed by modifications of the agonist NMe-F-K-P-dCha-L-r, in which the molecule was progressively substituted at position 73 with substituents of increasing size, Cha, F, Nph and W, which had the effect of reducing agonist activity. Receptor-binding assays, performed on isolated human neutrophil membranes, showed that the antagonist has 0.04% relative affinity of C5a for the receptor (Konteatis *et al*, 1994; DeMartino *et al*, 1995). A key feature of the reports by Springer et al is the definition of the binding of **1** (NMe-Phe-Lys-Pro-dCha-Trp-dArg-CO_2H) to the C5a receptor. These authors state that the C-terminal arginine is essential for receptor binding and antagonist activity. This is also the case in all the reports of agonist activity by small peptide analogues of the C-terminus of C5a. However, for the antagonist **1**, Springer et al go further and state that *the C-terminal carboxylate is an essential requirement for antagonist activity and receptor binding*. They proposed that the requirement of the carboxylate is probably the result of its specific interaction with an arginine (Arg 206) in the receptor (De Martino *et al*, 1995). This idea was supported by a great reduction in receptor-affinity for an analogue of **1** in which the D-arginine (NH_2-CH(CO_2H)-$(CH_2)_3NHC(:NH)NH_2$) was replaced by agmatine (NH_2-CH_2-$(CH_2)_3NHC(:NH)NH_2$). In summary, Springer and colleagues claim that the D-arginine interacts via its guanidinium side chain with a negatively charged amino acid side chain in the receptor *and* via interaction between the negatively charged C-terminal carboxylate of **1** and a positively-charged side chain residue in the receptor.

We have now determined the solution structure of this hexapeptide, and have surprisingly found that a terminal carboxylate group is not required for binding to C5aR or for antagonist activity, and that instead an unusual turn conformation is responsible for the activity. The hexapeptide and several new structurally related antagonists have been examined for both their receptor-binding affinities and antagonist activity, using intact polymorphonuclear (PMN) cells. Our results show that there is a hitherto unknown specific structural requirement for the binding of C5a to its receptor, which we believe to be common to ligands for the G protein-coupled receptor family. Our establishment of this specific structural requirement has enabled us to design and develop improved molecular probes of the complement system and of C5a-based drugs, and to design small molecules that target other G protein-coupled receptors, which are becoming increasingly recognised as important drug targets due to their crucial roles in signal transduction (G Protein-Coupled Receptors, IBC Biomedical Library Series, 1996).

Thus our results have enabled us to design constrained structural frameworks which enable hydrophobic groups to be assembled into a hydrophobic array for interaction with a G protein-coupled receptor, such as Site 2 of the C5a receptor as illustrated in Figure 1. Such frameworks, which may be cyclic or acyclic, have not heretofore been suggested for G protein-coupled receptor antagonists, such as antagonists of C5a.

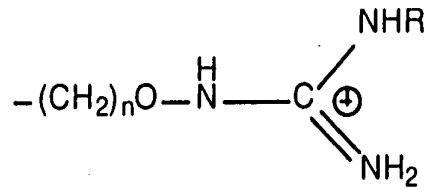
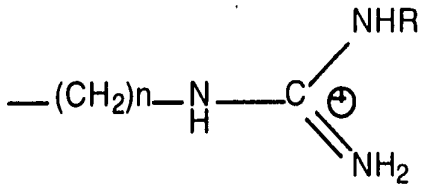
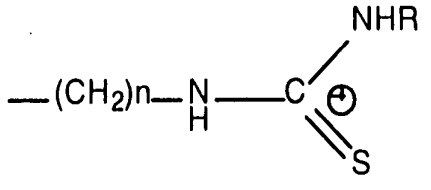
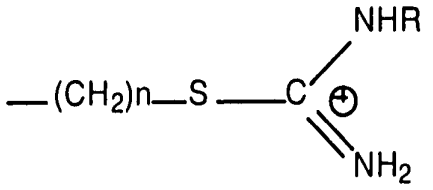
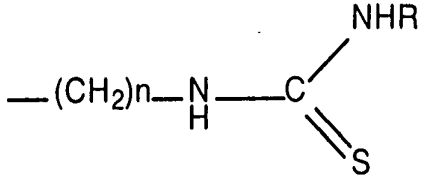
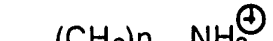
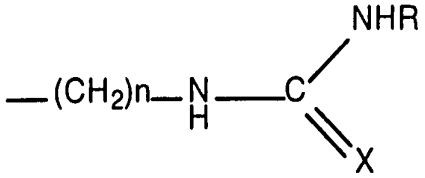
SUMMARY OF THE INVENTION

According to a first aspect, the invention provides an antagonist of a G protein-coupled receptor, which has no agonist activity, and which has a structure adapted to provide a framework of approximate dimensions as follows:



where the critical amino acid side chains are designated by A, B, C and D, or are as defined below.

A is any common or uncommon, basic, charged amino acid side chain which serves to position a positively charged group in this position, including, but not limited to the following side chains and other mimetics of arginine side chains:

where

X is H, NCN, NNO_2, $CHNO_2$ or NSO_2NH_2;

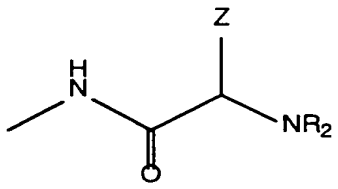
n is an integer from 1 to 4, and

R is H or an alkyl, aryl or other modified group such as CN, NH_2 or OH.

B is any common or uncommon, aromatic amino acid side chain which serves to position an aromatic side-chain in this position, including but not limited to the indole, indole methyl, benzyl, phenyl, naphthyl, naphthyl methyl, cinnamyl group or any other derivatives of these aromatic groups;

C is any common or uncommon, hydrophobic amino acid side chain which serves to position any alkyl, aromatic or other group in this position. Including, but not limited to D- or L-cyclohexyl alanine, leucine, valine, isoleucine, phenylalanine, tryptophan, methionine.

D is any common or uncommon, aromatic amino acid which serve to position an aromatic side-chain in this position, and has the structure:



where

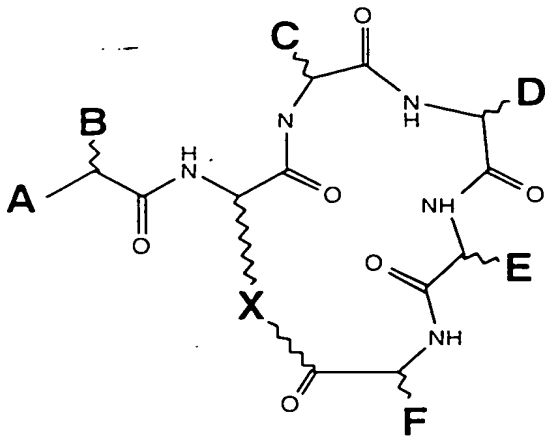
Z includes but is not limited to indole, indole methyl, benzyl, benzene, naphthyl, naphthyl methyl, or any other derivatives of these aromatic groups, and

R is H or any alkyl, aromatic, acyl or aromatic-acyl group including, but not limited to Me, ethyl, propyl, butyl, $-CO-CH_2CH_3$, $-CO-CH_3$, $-CO-CH_2CH_2CH_3$, $-CO-CH_2Ph$, or -CO-Ph.

Other cyclic or non-cyclic molecules, which may be peptidic or non-peptide in nature, can similarly be envisaged to support groups such as A, B, C and D for

interaction with a C5a receptor or other G protein-coupled receptor.

In one preferred embodiment, the antagonist has antagonist activity against C5a, and has no agonist activity against C5a, and has general formula:



where

A is H, alkyl, aryl, NH_2, NHalkyl, $N(alkyl)_2$, NHaryl or NHacyl;

B is an alkyl, aryl, phenyl, benzyl, naphthyl or indole group, or the side chain of a D- or L-amino acid selected from phenylalanine, homophenylalanine, tryptophan, homotryptophan, tyrosine, and homotyrosine,

C is the side chain of a D-, L- or homo-amino acid selected from proline, alanine, leucine, valine, isoleucine, arginine, histidine, aspartate, glutamate, glutamine, asparagine, lysine, tyrosine, phenylalanine, cyclohexylalanine, norleucine, tryptophan, cysteine and methionine;

D is the side chain of a D- or L-amino acid selected from cyclohexylalanine, homocyclohexylalanine, leucine, norleucine, homoleucine, homonorleucine and tryptophan;

E is the side chain of a D- or L-amino acid selected from tryptophan and homotryptophan;

F is the side chain of a D- or L-amino acid selected from arginine, homoarginine, lysine and homolysine; and

X is $-(CH_2)_nNH-$ or $(CH_2)_n-S-$, where n is an integer of from 1 to 4, preferably 2 or 3, $-(CH_2)_2O-$, $-(CH_2)_3O-$, $-(CH_2)_3-$, $-(CH_2)_4-$, or $-CH_2COCHRNH-$, where R is the side chain of any common or uncommon amino acid.

For the purposes of this specification, the term "alkyl" is to be taken to mean a straight, branched, or cyclic, substituted or unsubstituted alkyl chain of 1 to 6, preferably 1 to 4 carbons. Most preferably the alkyl group is a methyl group. The term "acyl" is to be taken to mean a substituted or unsubstituted acyl of 1 to 6, preferably 1 to 4 carbon atoms. Most preferably the acyl group is acetyl. The term "aryl" is to be understood to mean a substituted or unsubstituted homocyclic or heterocyclic aryl group, in which the ring preferably has 5 or 6 members.

According to a second aspect, the invention provides a pharmaceutical composition, comprising a compound according to the invention together with a pharmaceutically-acceptable carrier or excipient.

The compositions of the invention may be formulated for oral or parenteral use, but oral formulations are preferred. It is expected that most if not all compounds of the invention will be stable in the presence of digestive enzymes.

Suitable formulations for administration by any desired route may be prepared by standard methods, for example by reference to well-known text such as Remington; The Science and Practice of Pharmacy, Vol. II, 1995 (19^{th} edition), A.R. Gennaro (ed), Mack Publishing Company, Eastern Pennsylvania, or Australian Prescription Products Guide, Vol. 1, 1995 (24^{th} edition) J. Thomas (ed), Australian Pharmaceutical Publishing Company Ltd, Victoria, Australia.

In a third aspect the invention provides a method of treatment of a pathological condition mediated by G protein-coupled receptors, comprising the step of administering an effective amount of a compound of the invention to a mammal in need of such treatment.

Preferably the condition mediated by G protein-coupled receptors is a condition involving overexpression or underregulation of C5a. Such conditions include but are not limited to rheumatoid arthritis, adult respiratory distress syndrome (ARDS), systemic lupus erythematosus, tissue graft rejection, ischaemic heart disease, reperfusion injury, septic shock, psoriasis, gingivitis, atherosclerosis, Alzheimer's disease, lung injury and extracorporeal post-dialysis syndrome.

While the invention is not in any way restricted to the treatment of any particular animal or species, it is particularly contemplated that the compounds of the invention will be useful in medical treatment of humans, and will also be useful in veterinary treatment, particularly of companion animals such as cats and dogs, livestock such as cattle, horses and sheep, and zoo animals, including large bovids, felids, ungulates and canids.

The compounds may be administered at any suitable dose and by any suitable route. Oral administration is preferred because of its greater convenience and acceptability. The effective dose will depend on the nature of the condition to be treated, and the age, weight, and underlying state of health of the individual treatment. This will be at the discretion of the attending physician or veterinarian. Suitable dosage levels may readily be determined by trial and error experimentation, using methods which are well known in the art.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the two-site model for binding of C5a to its G protein-coupled receptor, C5R. The

black rods represent α-helical regions, and the open cylinders represent the transmembrane helices.

Figure 2 shows stacked plots of ^{1}H-NMR spectra, showing time-dependent decay of amide NH resonances for Trp (8.1ppm) and d-Cha (7.9 ppm) residues of **1** in d_6-DMSO containing D_2O after 10 minutes (bottom) and then 25, 40, 55, 70, 130, 190, 250, 385 and 520 minutes.

Figure 3 shows backbone C, N, O atoms of twenty lowest energy minimized NMR structures of **1** in d_6-DMSO at 24°C) .

Figure 4 shows a schematic representation of H-bonding in the structure of **1** from NMR spectra in d_6-DMSO.

Figure 5 shows (a) receptor binding, as indicated by inhibition of binding of ^{125}I-C5a to human PMNs by **1** (●); **2** (Δ); **3** (▲); **6** (O). (b) C5a antagonist potency as shown by inhibition of myeloperoxidase (MPO) release from human PMNs by : **1** (■, n= 9) and **6** (▲, n=4). All data are means ± SEM.

Figure 6 shows receptor binding of cyclic C5a antagonists, as shown by inhibition of binding of ^{125}I-C5a to human PMNs (n=5).

Figure 7 shows superimposed structures of **1** (light, NMR structure) and **6** (dark, computer modelled structure). Phe and Trp side chains are omitted from **6** for clarity.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described by way of reference only to the following general methods and experimental examples. Abbreviations used herein are as follows:

BOP	benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
D-Cha	D-cyclohexylamine
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethylsulphoxide

HBTU	O-benzotriazole N',N',N',N'-tetramethyluronium hexafluorophosphate;
PMN	polymorphonuclear granulocyte
RP-HPLC	reverse phase-high performance liquid chromatography
TFA	trifluoroacetic acid;

General Methods

Protected amino acids and resins were obtained from Novabiochem. TFA, DIEA and DMF (peptide synthesis grade) were purchased from Auspep. All other materials were reagent grade unless otherwise stated. Preparative scale reverse-phase HPLC separations were performed on a Vydac C18 reverse-phase column (2.2 x 25 cm), and analytical reverse-phase HPLC separations were performed on a Waters Delta-Pak PrepPak C18 reverse-phase column (0.8 x 10 cm), using gradient mixtures of solvent A = water/0.1% TFA and solvent B = water 10%/acetonitrile 90%, 0.09% TFA. The molecular weight of the peptides was determined by electrospray mass spectrometry recorded on a triple quadrupole mass spectrometer (PE SCIEX API III), as described elsewhere (Haviland *et al*, 1995). ^{1}H-NMR spectra were recorded on either a Bruker ARX 500 MHz or a Varian Unity 400 spectrometer. Proton assignments were determined by 2D NMR experiments (DFCOSY, TOCSY, NOESY).

Non-peptidic compounds were synthesized using conventional organic chemical methods. Compounds were analysed by ^{1}H-NMR spectroscopy and by mass spectrometry.

Peptide synthesis

Linear peptide sequences were assembled by manual step-wise solid-phase peptide synthesis with HBTU activation and DIEA *in situ* neutralisation. Boc chemistry was employed for temporary N^{α}-protection of amino acids with two 1 min. treatments with TFA for Boc group removal. The peptides were fully deprotected and cleaved by treatment with liquid HF (10 ml; p-cresol (1 ml); -5°C; 1-

2 hrs). Analytical HPLC (gradient; 0% B to 50% B over 40 min): **1**, Rt = 32.0 min., $[M+H]^+$(calc.) = 900.5, $[M+H]^+$(exper.) = 900.7; **2**, Rt = 32.2 min., $[M+H]^+$(calc.) = 899.6, $[M+H]^+$(exper.) = 899.7; **3**, Rt = 30.0 min., $[M+H]^+$(calc.) = 900.5, $[M+H]^+$(exper.) = 900.7; **4**, Rt = 23.8 min., $[M+H]^+$(calc.) = 860.5, $[M+H]^+$(exper.) = 860.5.

*a) Synthesis of cycle **5***

The linear peptide was synthesised by Fmoc chemistry using HBTU/DIEA activation on an Fmoc-D-Arg(Mtr)-Wang resin. Fmoc group removal was effected using two 1 min. treatments with 50% piperidine/DMF. Cleavage and deprotection using 95% TFA/2.5% TIPS/2.5% H_2O gave the Mtr-protected peptide, which was purified by RP-HPLC. Cyclization of the protected, purified peptide using 3eq BOP and 10eq DIEA at a 1 mM concentration in DMF stirring for 15 hrs gave the cyclised product, which was fully deprotected using 1M TMSBr in TFA. A final RP-HPLC purification gave the desired peptide in yields of 50% for the cyclisation. Rt = 37.7 min., $[M+H]^+$(calc.) = 910.5, $[M+H]^+$(exper.) = 910.7.

*b) Synthesis of cycle **6***

Cyclization of the cleaved and fully deprotected peptide was achieved by stirring a 1mM solution in DMF with 3eq BOP and 10eq pyridine as base for 15hrs. A final RP-HPLC purification gave the desired peptide in yields of 22% for the cyclisation. Rt = 37.3 min., $[M+H]^+$(calc.) = 896.5, $[M+H]^+$(exper.) = 896.5.

NMR Structure Determination

^{1}H-NMR spectra were recorded for compound **1** (3 mg in 750 µl d_6-DMSO, δ 2.50) referenced to solvent on a Varian Unity 400 spectrometer at 24°C. Two dimensional ^{1}H-NMR NOESY (relaxation delay 2.0s, mix time 50-300 ms), DFQ- COSY and TOCSY (mixing time 75ms) experiments were

acquired and recorded in phase sensitive mode. Acquisition times = 0.186 s, spectral width = 5500Hz, number of complex points (t_1 dimension) = 1024 for all experiments. Data was zero-filled and Fourier transformed to 1024 real points in both dimensions.

NMR data was processed using TRIAD software (Tripos Assoc.) on a Silicon Graphics Indy work station. 2D NOE cross peaks were integrated and characterised into strong (1.8-2.5Å), medium (2.3-3.5Å) and weak (3.3-5.0Å). Preliminary three-dimensional structures were calculated from upper and lower distance limit files using Diana 2.8 (69 distance constraints, including 27 for adjacent residues and 6 further away) with the redundant dihedral angle constraints (REDAC) strategy. Upper and lower distance constraints were accurately calculated using MARDIGRAS. At this stage the peptide was examined for possible hydrogen bonds, and these were added as distance constraints. The 50 lowest energy Diana structures were subjected to restrained molecular dynamics (RMD) and energy minimisation (REM). Initially, REM consisted of a 50 step steepest descent followed by 100 step conjugate gradient minimisation. RMD was performed by simulated heating of the structures to 300K for 1ps, followed by 500K for 1ps. The temperature was gradually lowered to 300K over 2ps and finally for 2ps at 200K. REM was performed again with a 50 step steepest descent, 200 step conjugate gradient followed by a 300 step Powell minimisation. The final structures were examined to obtain a mean pairwise rms difference over the backbone heavy atoms (N, Cα and C). Twenty of the 50 structures had a mean rmsd < 0.5 Å for all backbone atoms (O, N, C).

Molecular Modelling

A model of cycle **6**, shown in Figure 7, was created from the NMR structure of **1** by deleting all NMR constraints, fusing the ornithine side chain amine to the C-terminal carboxylate of d-Arg to form an amide, and

minimising using Powell forcefield (1000 iterations). The modelled structure was then superimposed on the NMR structure with an rmsd 0.224Å.

Receptor-Binding Assay

Assays were performed with fresh human PMNs, isolated as previously described (Sanderson *et al*, 1995), using a buffer of 50 mM HEPES, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5% bovine serum albumin, 0.1% bacitracin and 100 µM phenylmethylsulfonyl fluoride (PMSF). In assays performed at 4°C, buffer, unlabelled human recombinant C5a (Sigma) or peptide, Hunter/Bolton labelled ^{125}I-C5a (~ 20 pM) (New England Nuclear, MA) and PMNs (0.2×10^6) were added sequentially to a Millipore Multiscreen assay plate (HV 0.45) having a final volume of 200 µL/well. After incubation for 60 min at 4°C, the samples were filtered and the plate washed once with buffer. Filters were dried, punched and counted in an LKB gamma counter. Non-specific binding was assessed by the inclusion of 1 mM peptide or 100 nM C5a which typically resulted in 10-15% total binding.

Data was analysed using non-linear regression and statistics ($p < 0.0001$) with Dunnett post test.

Myeloperoxidase Release

Cells were isolated as previously described (Sanderson *et al*, 1995a) and incubated with cytochalasin B (5µg/mL, 15 min, 37°C). Hank's Balanced Salt solution containing 0.15% gelatin and peptide was added on to a 96 well plate (total volume 100 µL/well), followed by 25 µL cells (4×10^6/mL). To assess the capacity of each peptide to antagonise C5a, cells were incubated for 5 min at 37°C with each peptide, followed by addition of C5a (100 nM) and further incubation for 5 min. Then 50 µL of sodium phosphate (0.1M, pH 6.8) was added to each well, the plate was cooled to room temperature, and 25 µL of a fresh mixture of equal volumes of dimethoxybenzidine (5.7 mg/mL)

and H_2O_2 (0.51%) was added to each well. The reaction was stopped at 10 min by addition of 2% sodium azide. Absorbances were measured at 450 nm in a Bioscan 450 plate reader, corrected for control values (no peptide), and analysed by non-linear regression.

***In Vivo* Assays of Anti-Inflammatory Activity**

The following well-known *in vivo* assay systems may be used to assess the anti-inflammatory activity of compounds of the invention. All assay data are analysed using non-linear regression analysis and Student's t-test, with $p<0.05$ as the threshold level of significance.

Carrageenan Paw Oedema

Anaesthetised (i.p. ketamine & xylazine) Wistar rats (150-200g) or mice were injected with sterilised air (20ml day 1, 10mls day 4) into the subcutaneous tissue of the back. The cavity can be used after 6 days, whereupon carrageenan (2ml, 1% w/w in 0.9% saline) was injected into the air pouch and exudate was collected after 10 hr. Test compounds are administered daily after Day 6 and their anti-inflammatory effects assayed by differential counting of cells in the air-pouch exudate. Rats were killed at appropriate times after injection and 2ml 0.9% saline was used to lavage the cavity, lavage fluids were transferred to heparinised tube and cells were counted with a haemocytometer and Diff-Quik stained cytocentrifuged preparation.

Alternatively, a routine carrageenan paw oedema was developed in Wistar rats by administering a pedal injection of carrageenan to elicit oedema which is visible in 2h and maximised in 4h. Test compounds are given 40 min before inflammagen and evaluated by microcaliper measurements of paws after 2 & 4 hrs. See Fairlie,D.P. *et al* (1987). Also see Walker and Whitehouse (1978).

Adjuvant Arthritis.

Adjuvant arthritis was induced in rats (3 strains) either microbially (injection of heat-killed *Mycobacterium tuberculosis*) or chemically (with avridine) by inoculation with the arthritogenic adjuvant co-administered with oily vehicles (Freund's adjuvants) in the tail base. (See Whitehouse, M. W., Handbook of Animal Models for the Rheumatic Diseases, Eds. Greenwald, R. A.; Diamond, H. S.; Vol. 1, pp. 3-16, CRC Press)

Within 13 days the adjuvant arthritis is manifested by local inflammation and ulceration in the tail, gross swelling of all four paws, inflammatory lesions in paws and ears, weight loss and fever. These symptoms, which are similar to those of inflammatory disease in humans (Winter and Nuss, 1966), can be alleviated by agents such as indomethacin or cyclosporin which also show beneficial effects in man (*eg.* Ward and Cloud, 1966). Without drug treatment at Day 14, arthritic rats had hypertrophy of the paws, reduced albumin but raised acute phase reaction proteins in serum, and depressed hepatic metabolism of xenobiotics as indicated by prolonged barbiturate-induced sleeping times.

To assess activity, compounds were administered for 4 days orally (≤10mg/kg/day) or i.p. from Days 10-13 following inoculation with arthritogen (Day 0). The inflammation was either not visible or very significantly reduced in rear or front paws as assessed by microcaliper measurements of paw thickness and tail volume, as well as by gross inspection of inflammatory lesions. Animals are sacrificed by cervical dislocation on Day 18 unless arthritis signs are absent, whereupon duration of observations is continued with special permission from the Ethics committees. Experiments are staggered to maximise throughput and allow early comparisons between compounds. This routine assay is well-accepted in identifying anti-inflammatory agents for use in humans.

Example 1 Structure-Activity Relationship of CSA Agonists

We have focussed on the C-terminal residues of C5a, in order to explore structure-activity relationships in the search for peptide sequences with potent agonist activity. Many of these peptides are full agonists relative to C5a, but have markedly lower potency (Sanderson *et al*, 1994; Sanderson *et al*, 1992; Finch *et al*, 1997). Our initial structure-activity investigations have been particularly informative. Mutating the decapeptide C-terminus of C5a (peptide 1, $C5a_{65-74}$, ISHKDMQLGR) twice with $I_{65}Y$ and $H_{67}F$ (*eg.* peptide 2) led to enhancement of agonist potency by about 2 orders of magnitude. These results are summarised in Table 1. Analyses of Ramachandran plots and 2D NMR spectra for #2 suggested that certain structural features, namely a twisted "helix-like" backbone conformation for residues 65-69 and a ß-turn for residues 71-74, might be responsible for activity. These preliminary results provided some insight to structural requirements for tight binding to a C5a receptor.

Table 1

Pharmacological Activity of New C5a Agonist Analogues*

Peptide No.	Peptide	Fet, Art EC_{50} (μM)	PMN Enz Release EC_{50} (μM)	Bind Aff IC_{50} (μM)
1	$C5a_{65-74}$ (ISHKDMQLGR)	>1000	>1000	>1000
2	YSFKDMQLGR	9.6	92	1.3
10	YSFKDMPLaR	0.5	72	3.7
54	YSFKPMPLaR	0.2	4.1	6.0
74	$C5a_{37-46}$-ahxYSFKPMPLaR	0.06	5.9	0.7
87	$C5a_{12-20}$-ahxYSFKPMPLaR	0.08	0.7	0.07
	C5a	0.02	0.03	0.0006

* Finch *et al*, 1997

Compounds 54, 74 and 87 in Table 1 are the highest affinity small C5a agonists so far known, with up to 25% C5a potency in human fetal artery, 5% C5a potency in human PMN enzyme release assays and 1% C5a affinity for PMN C5aR (Kawai *et al*, 1991). For the PMN receptor, these compounds have up to 100-fold higher affinity than any agent previously described in the literature.

The "high" affinities (70nM-6μM) of our new agonist analogues for C5aR in intact PMN cells have enabled us to identify a common topographical feature in peptide agonists that correlates with expression of spasmogenic activities and enzyme-release assays in human PMNs. This preferred backbone conformation is a type II ß-turn.

The small size of our agonist peptides makes them amenable to synthetic modification to optimise their affinities, activities, and bioavailabilities, and hence useful as mechanistic probes of receptor activation.

Example 2 NMR Structure of C5a Antagonist

We used two dimensional Nuclear Magnetic Resonance spectroscopy to determine the three dimensional structure of **1** and find that while there is no discernible structure in water, there is evidence of a stable gamma-turn structure in dimethylsulfoxide.

The 1D ^{1}H-NMR spectrum of peptide **1** in d_6-DMSO at 24°C shows 4 distinct resonances for amide-NH protons, as summarized in Table 2. To establish their possible involvement in intramolecular hydrogen bonds, a deuterium exchange experiment was performed by adding a 10-fold excess of D_2O to the solution. Two of the amide-NH doublets disappeared immediately, along with resonances attributable to the N-terminal methylamine protons. However, the other two amide NH resonances, as well as a broad resonance at approximately 8.05 ppm, persisted for up to 6.5 hours (Figure 2). These three slowly-exchanging protons are assigned to the amide NHs of Trp and d-Cha and the side chain amine of Lys, the slow exchange behaviour

being characteristic of hydrogen-bonding. The amine assignment was established from the TOCSY spectrumm where cross peaks were observed between the protonated amine and the ε, δ and γ CH_2 protons. A temperature dependence study (20-60°C) of the amide-NH chemical shifts ($\Delta\delta/T$ = 2.5 ppb/deg, dCha-NH; 6 ppb/deg, Trp-NH; 6.5 ppb, Lys-NH; 8.7ppb, Arg-NH) unambiguously confirmed the involvement of the dCha-NH only in intramolecular hydrogen bonding.

Table 2

^{1}H-NMR Assignments[a] for **1** in d_6-DMSO

Residue	[b]HN	Hα	Hβ	Hγ	Others
MePhe	-	4.06	3.09, 3.06	-	[c] 7.17, 7.29; [d]2.46; [f]8.98
Lys	8.83	4.54	1.74, 1.55	1.32	[c]1.51; [f]2.74, [g]7.76 (NH_2)
Pro	-	4.30	2.084, 1.74	1.88, 1.78	[e]3.61, [f]3.48
d-Cha	7.91	4.35	1.19, 1.06	0.76	[c]1.43, 1.08; [f]1.61, 1.58; 0.73
Trp	8.01	4.65	3.11, 2.94	-	[c]6.97,7.06,7.13,7.32,7.65; [g]10.80
d-Arg	8.44	4.20	1.73, 1.58	1.42	[e]3.08; [g]7.60

a Referenced to residual d_5-DMSO at 2.50 ppm.

b Amide NHs, $^3J_{NH\text{-}C\alpha H}$ values (Hz) : 7.91 (Lys), 7.77 (d-Arg), 8.34 (Trp), 8.53 (d-Cha).

c Aromatics

d N-Me.

e Hδ.

f Hε

g NH/NH_2 amine.

A series of 2D ^{1}H-NMR spectra were measured for **1** at 24°C in d_6-DMSO to determine the three-dimensional structure. TOCSY and DFQ-COSY experiments were used to identify residue types, while sequential assignments were made from analysis of NOESY data. From a series of 100 structures generated from NOESY data, fifty of the lowest energy structures were subjected to restrained molecular dynamics (200K-500K) and energy minimised. A set of 20 calculated structures with a root mean square deviation (rmsd) < 0.5Å (backbone atoms) are superimposed in Figure 3, and clearly depict a turn conformation.

In combination, the NMR constraint data, $^3J_{NH-C\alpha H}$ values, deuterium exchange and temperature dependence data establish an unusual turn structure for hexapeptide **1** which is constrained by up to three hydrogen bonds, as shown in Figure 4. The evidence is very strong for *one intramolecular hydrogen bond* from dCha-NH....OC-Lys (2.72 Å, N-H..O angle 157°, C=O...H angle 84°), forming a 7-membered ring that defines an inverse γ-turn. The dChaNH-O-TrpNH angle is 56.4°. The deuterium exchange data and NMR constraint data together point to a *second intramolecular hydrogen bond* Trp-NH....OC-Lys (3.31 Å, N-H..O angle 159°, CO...H angle 137.3°) forming a 10-membered ring characteristic of a β-turn. The ϕ and ψ angles (ϕ_2=-58.4°, ψ_2=62.0°; ϕ=96.6°, ψ_3=16.6°) most closely match a type II β-turn (Bandekar, 1993; Hutchinson and Thornton, 1994) which is distorted by the presence of the γ-turn wholly *within* the β-turn.

To our knowledge this is the first example of an intramolecular hydrogen bond between residues within a β-turn, although there are many examples of hydrogen bonds between a residue within the "10 membered ring" of a β-turn and a residue outside of it (Bandekar, 1993). *A third hydrogen bond* (2.76Å, N-H...O angle 160.3°), between the side-chain amine of Lys and the C-terminal carboxylate, is suggested by the NMR constraint data, by slow NH/ND exchange and by detection of a weak NOE between Lys-

NH...Trp-αCH_2. This may further constrain the molecule into the observed turn conformation. Such ion-pairing is common in dipolar aprotic solvents such as dimethylsulphoxide and may also be relevant in a hydrophobic protein environment.

NMR solution structures have also been determined for several of the cyclic antagonists described in the following examples, and show that in each case the type II β-turn is preserved and stabilized by the cyclic structure.

The constraining β and γ turns proposed in the linear peptide **1** have parallels in cyclic peptides. We have previously detected overlapping β and γ turns in a cyclic octapeptide from ascidiacyclamide (Abbenante *et al*, 1996). Combinations of a β- and γ-turn have also been found in the backbones of cyclic penta- and hexapeptides, particularly those containing alternating D- and L-amino acids (Marraud *et al*, 1996; Fairlie *et al*, 1995; Kessler *et al*, 1995; Stradley *et al*, 1990). For example a type II β-turn and an inverse γ-turn have been identified in cyclic antagonists c-(D- Glu-Ala-D-allo-Ile-Leu-D-Trp] (Ihara *et al*, 1991; Coles *et al*, 1993; Ihara *et al*, 1992; Bean *et al*, 1994) and c-(D-Asp-Pro-D-Val-Leu-D-Trp) (Bean *et al*, 1994) for endothelin receptors, and in members of the rhodopsin family of G protein-coupled receptors with seven transmembrane domains (X.-M.Cheng *et al*, 1994). In the latter case, as in **1**, an inverse γ-turn forms between residues (Asp-CO.....Val-NH, Lys-CO....dCha-NH) that flank the proline.

Example 3 Structure-Activity Relationships

We also examined the receptor-binding and antagonist activity of the hexapeptide **1** for comparison with our compounds. The previous report by Konteatis *et al* (1994) concerned the ability of **1** to compete with C5a binding to receptors on isolated PMN membranes (IC_{50} 70 nM), which is not necessarily physiologically relevant. We examined competition between **1** and C5a using intact PMN

cells, and found that, under these conditions, **1** binds with much lower receptor affinity of IC_{50} 1.8 μM. We confirmed that **1** is a full antagonist with no agonist properties. These results are summarized in Figure 5a and Table 3. The *relative* affinity (ratio) of **1** for the C5aR in intact PMNs in our assays was similar to that previously reported for isolated PMN membranes.

We have also found that **1** shows antagonist activity against both C5a (Figure 5b) and a C-terminal agonist decapeptide analogue **7** (YSFKPMPLaR) (Finch *et al*, 1997) of the C-terminus $C5a_{65-74}$, suggesting that it acts on site 2 of the receptor. Compounds **1** and **7** have similar μM affinity for the receptor C5aR on intact polymorphonuclear leukocytes, as shown in Table 3.

An important new discovery from the data in Table 3 is the excellent linear correlation ($r = 0.93$) between the log of binding affinities and the log of antagonist potencies for these Site 2 antagonists (compounds **1-6**, Table 3). The importance of this linear relationship is that since receptor affinity and antagonist activity are directly proportional, the experimentally simpler approach of measuring receptor binding can be generally used to estimate the antagonist activity for such small compounds, provided that there is no evidence of agonist activity.

Table 3

Receptor-binding Affinities[a] and Antagonist Activities[b] in Human PMNs

	Compound	Receptor Affinity[a] IC_{50} (μM)	Antagonist Potency[b] IC_{50} (μM)	Agonist Activity[c]
1	MeFKP(dCha)Wr	1.8 (15)	0.085 (9)	No
2	MeFKP(dCha)Wr-$CONH_2$	14 (5)	0.5 (3)	No
3	MeFKP(dCha)WR	11 (5)	0.7 (3)	No
4	MeFKPLWR	144 (1)	>1000 (3)	nd
5	Ac-F-c[KP(dCha)Wr]	3.2 (40	0.090 (5)	No
6	Ac-F-c[OP(dCha)Wr]	0.28 (6)	0.012 (4)	No
7	YSFKPMPLaR	6.0[d]	-	Yes
8	$C5a_{65-74}$, ISHKDMQLGR	>1000[e]	-	-
9	C5a	0.0008 (9)	-	Yes

No. of experiments in parenthesis. Corrected for amino acid content. nd=not determined

[a] 50% reduction in binding of ^{125}I-C5a to intact human PMNs

[b] 50% reduction in myeloperoxidase secretion from human PMNs mediated by 100 nM C5a

[c] Agonist activity in dose range 0.1 nM-1 mM

[d] Finch *et al*, 1997

[e] Kawai *et al*, 1991

It has previously been proposed that the C-terminus of C5a and of agonist peptides is essential for activity, due to its interaction with a positively charged Arg209 of the receptor (Konteatis *et al*, 1994; DeMartino *et al*, 1995). We confirm here that the C-terminal carboxylate is indeed important for activity (**2** vs. **1**), but wondered whether the origin of this effect might be due to hydrogen bonding between the carboxylate anion and the positively charged amine side chain of Lys. Conversion to the amide (**2**) certainly reduces both receptor-affinity and antagonist activity approximately 5-fold. Changing chirality of the Arg-Cα (**3** vs. **1**) causes a similar reduction in activity, and replacing dCha with the less bulky Leu residue (**4**) is also detrimental to receptor binding. However, potency is recovered for cyclic compounds **5** and **6**, in which an amide bond is tolerated at the C-terminus, consistent with the structural interpretation above that the advantage of the carboxylate in **1** may be associated with intramolecular hydrogen bonding. The replacement of this hydrogen bond in **1** with a covalent amide bond in **5** and **6** more effectively stabilizes the turn conformation.

Example 4 Cyclic Antagonists of C5a

Some examples of these cyclic antagonists and their receptor-binding affinities and antagonist potencies are given in Table 3 and Table 4 and Figures 5 and 6. These results demonstrate:

(1) that the cyclic molecules have higher receptor-affinity and are more potent antagonists than acyclic (linear) peptides,

(2) that one of the two possible cyclic diastereomers is consistently favoured for binding to the C5a receptor,

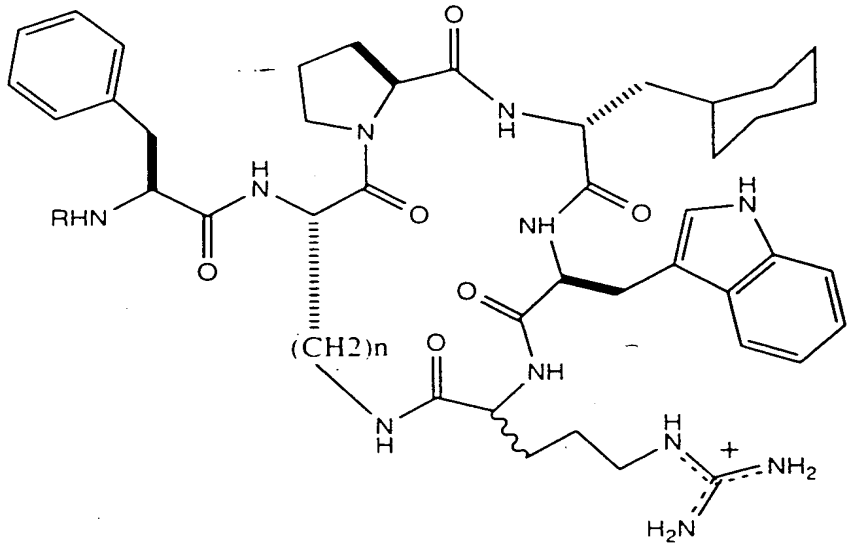
(3) that the cycles have an optimum ring size for receptor-binding,

(4) that there is a linear relationship between log (antagonist potency) and log (receptor affinity).

Table 4 lists the C5a receptor affinities of some examples of cyclic antagonists of C5a, and their ability to bind to inhibit binding of C5a to human PMNc is illustrated in Figure 6. Surprisingly these data show that the L-arginine is preferred over the D-arginine, in contrast to the linear compound **1** in which the D-arginine confers higher affinity for the receptor. The data also shows that the size of the macrocycle is optimal when n = 2 or 3, the smaller cycle where n = 1 and the larger cycle when n = 4 being clearly less active. This requirement for a tightly constrained cycle is probably due to the need to correctly position the attached side chain residues of, for example, Trp, dCha, Arg and Phe for interaction with the receptor.

Table 4

Receptor Binding and Antagonist Activity of Cyclic Molecules



Compound	n	R	Isomer*	Receptor Affinity μM	Agonist Activity
10	1	H	S-	9	No
11			R-	34	No
12	2	H	S-	0.3	No
13			R-	3.7	No
14	3	Ac	S-	0.3	No
15		Ac	R-	38	No
16	4	Ac	S-	3.2	No
17		Ac	R-	51	No

* Refers to stereochemistry of Arg side chain.

Example 5 Computer Modelling of Antagonist Structures

Figure 7 compares the computer-modelled structure of the cyclic antagonist **6** with the NMR solution structure presented above for the acyclic antagonist **1**. These backbone structures are strikingly similar, and strongly suggest that the receptor-binding conformations of these molecules involve the same turn structure. Compound **6**, a more potent antagonist than **5**, also has a shorter linker, which tightens the turn and slightly alters the conformational space accessible to the key side chains of Phe, dCha, Trp and Arg. The conformational limitations placed on the hexapeptide derivative **6** by the cycle are responsible for a $\geq 10^4$ increase in receptor-binding affinity over the conformationally flexible decapeptide C-terminus of C5a (**8**, Table 3).

There is an excellent correlation ($r = 0.93$) between binding affinities and antagonist potency for the site 2 antagonists (compounds **1-6**, Table 3). It thus appears that antagonist potency is dependent upon changes that occur at site 2 alone. Without wishing to be bound by any proposed mechanism, we believe that this may be because the mechanism of antagonism is related to conformational change induced by **1** at site 2 of the receptor.

Example 6 Characterisation of C5aRs on Different Cells

Currently there is no information about different types of C5aRs. We have shown marked differences in the responsiveness of different cells containing functional C5aRs to these agonists (Sanderson *et al*, 1994, 1995; Finch *et al*, 1997) and we can now gather more information by examining potency and efficacy of selective agonists and antagonists relative to human recombinant C5a. For agonists, the tissue or cell selectivity may reveal potentially different receptor subtypes. Binding assays using human PMNs, U937 cells, or circulating monocytes are used to determine affinities for C5aRs. Selectivity for

different C5aRs is ascertained by differential antagonism and pA2 values. This combined approach allows pharmacological characterisation of new agonists or antagonists, and may lead to a potential classification of C5aR subtypes on different cells.

Example 7 *In Vivo* Activity of Cyclic C5a Antagonists

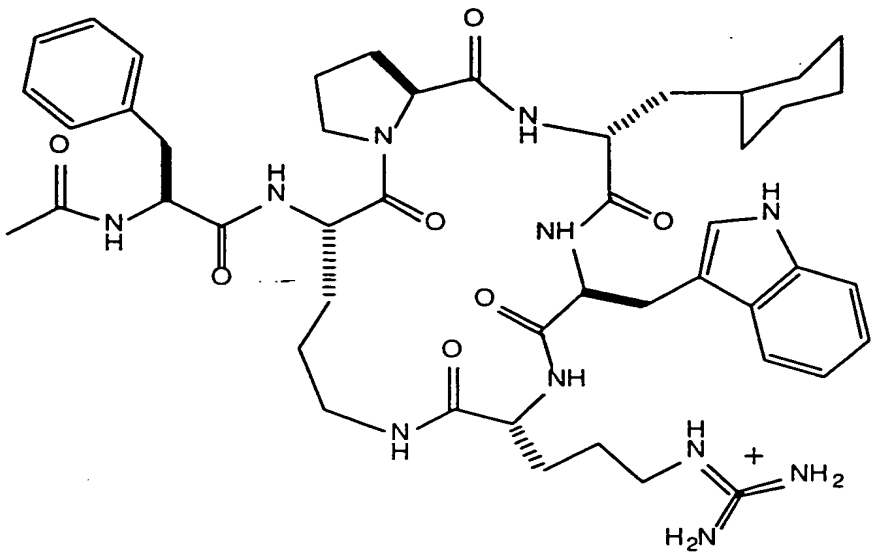
Preliminary experiments in rats have revealed that the cyclic antagonists summarized in Table 4 are active at less than 20 mg/kg as anti-inflammatory agents in supressing the onset of either carrageenan-induced paw oedema or adjuvant-induced polyarthritis. The effective dosages appear to be around 10 mg/kg given i.p. or p.o. Many current anti-inflammatory drugs used in humans were initially evaluated in such assays, and also showed activity in these rat models of inflammation. These preliminary indications of efficacy *in vivo* indicate that C5a antagonists may have therapeutic potential in human inflammatory conditions.

In recent years there have been many attempts to mimic β-turn peptides that represent bioactive protein surfaces, resulting in notable mimetics for RGD peptides, somatostatin and opioid peptides, to name a few derived through structure-activity relationships (see for example Marraud and Alebry, 1996; Fairlie *et al*, 1995). Most of these examples preserve the turn structure through cyclisation of the peptide. On the other hand there are comparatively few short acyclic peptides that have been found to have substantial turn structure in solution (Dyson *et al*, 1988; Rizo and Gierasch, 1992; Prêcheur *et al*, 1994). It is usually argued that short acyclic peptides adopt a myriad of solution structures that may include small populations of turn structures that are responsible for bioactivity. This invention describes a series of conformationally-constrained molecules that are

preorganized for binding to the same G protein-coupled receptor(s) of human cells that are targeted by human C5a. The invention is applicable to other G protein-coupled receptors.

The principal feature of the compounds of the invention is the preorganized arrangement which brings at least three hydrophobic groups and a charged group into neighbouring space, creating a hydrophobic surface 'patch'. These results enable the design and development of even more potent conformationally-constrained, small molecule antagonists of C5a.

In the light of the aforementioned prior art, it was surprising to find that a C-terminal carboxylate was not required in our compounds in order to obtain good receptor-binding or antagonist activity. The cyclic antagonists have an amide bond at the 'C-terminal' arginine position, consistent with the structural interpretation that the advantage of the carboxylate in **1** may be associated with intramolecular hydrogen bonding within the hydrophobic environment of the receptor. The replacement of this hydrogen bond in **1** with a covalent amide bond, as for example in **5**, **6** and **10** to **17** can more effectively stabilise the turn conformation.



Cyclic and non-peptidic antagonists have several important advantages over peptides as drugs. The cycles described in this invention are stable to proteolytic degradation for at least several hours at 37°C in human blood or plasma, as well as in human or rat gastric juices and in the presence of digestive enzymes such as pepsin, trypsin and chymotrypsin. In contrast, short peptides composed of L-amino acids are rapidly degraded to their component amino acids within a few minutes under these conditions. A second advantage lies in the constrained conformations adopted by the cyclic and non-peptidic molecules, whereas acyclic or linear peptides are flexible enough to adopt several structures in solution other than the required receptor-binding structure. Thirdly, cyclic and non-peptidic compounds are usually more lipid-soluble and more pharmacologically bioavailable as drugs than peptides, which can rarely be administered orally. Fourthly, the plasma half-lives of cyclic and non-peptidic molecules are usually longer than those of peptides.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

References cited herein are listed on the following pages, and are incorporated herein by this reference.

REFERENCES

Abbenante, G., Fairlie, D.P., Gahan, L.R., Hanson, G.R., Pierens, G.K. and van den Brenk, A.L.
J. Am. Chem. Soc., 1996 118 10384-10388

Bandekar, J.
Vib. Spectros., 1993 5 143-173

Bean, J.W., Peishoff, C.E. and Kopple, K.D.
Int. J. Protein Res., 1994 44 223

Coles, M., Sowemimo, V., Scanlon, D., Munro, S.L.A., Craik, D.J.
J. Med. Chem., 1993 36 2658

DeMartino, J.A., Konteatis, Z.D., Siciliano, S.J., Van Riper, G., Underwood, D.J., Fischer, P.A., Springer, M.S.
J. Biol. Chem., 1995 270 15966-15969

DeMartino, J.A., Van Riper, G., Siciliano, S.J., Molineaux, C.J., Konteatis, Z.D., Rosen, H. Springer, M.S.
J. Biol. Chem., 1994 269 14446-14450.

Drapeau, G., Brochu, S., Godin, D., Levesque, L., Rioux, F. and Marceau, F.
Biochem. Pharm., 1993 45 1289- 1299

Dyson, H.J., Rance, M., Hougten, R.A., Lerner, R.A. and Wright, P.E.
J. Mol. Biol., 1988 201 161-200

Ember, J.A., Sanderson, S.D., Taylor, S.M., Kawahara, M. and Hugli, T.E.
J. Immunol., 1992 148 3165-3173

Fairlie, D.P., Abbenante, G. and March, D.
Curr. Med. Chem., 1995 2 672-705

Fairlie, D.P., Whitehouse, M. and Broomhead, J.
Chem. Biol. Interact., 1987 61 277-291

Finch, A.M., Vogen, S.M., Sherman, S.A., Kirnarsky, L., Taylor, S.M., and Sanderson, S.D.
J. Med Chem., 1997 40 877

Hutchinson, E.G. and Thornton, J.M.
Protein Sci., 1994 3 2207-2216

Ihara, M., Fukuroda, T., Saeki, T., Nishikibe, M., Kojiri, K., Suda, H. and Yano, M.
Biochem. Biophys. Res. Comm., 1991 178 132-137

Ihara, M., Noguchi, K., Saeki, T., Fukuroda, T., Tsuchida, S., Kimura, S., Fukami, T., Ishikawa, K., Nishikibe, M., and Yano, M.
Life Sciences, 1992 50 247

Kawai, M., Quincy, D.A., Lane, B., Mollison, K.W., Luly, J.R., Carter, G.W.
J. Med. Chem., 1991 34 2068-71

Kawai, M., Quincy, D.A., Lane, B., Mollison, K.W., Or, Y.-S., Luly, J.R., and Carter, G.W.
J. Med. Chem., 1992 35 220-223

Kessler, H., Diefenbach, B., Finsinger, D., Geyer, A., Gurrath, M., Goodman, S.L., Hoelzemann, G., Haubner, R., Jonczyk, A. *et al*
Lett. Pept. Sci., 1995 2 155-160

Kohl, J., Lubbers, B., Klos, A., *et al.*
Eur. J. Immunol., 1993 23 646-652

Konteatis, Z.D., Siciliano, S.J., Van Riper, G., Molineaux, C.J., Pandya, S., Fischer, P., Rosen, H., Mumford, R.A., and Springer, M.S.
J. Immunol., 1994 153 4200- 4204

Marraud, M. and Aubry, A.
Biopolymers, 1996 40 45-83

Morgan, E.L., Sanderson, S.D., Scholz, W., Noonal, D.J., Weigle, W.O. and Hugli, T.E.
J. Immunol., 1992 48 3937-3942

Prêcheur, Bossus, M., Gras-Masse, H., Quiniou, E., Tartar, A. and Craescu, C.T.
J. Biochem., 1994 220 415-425

Rizo, J. and Gierasch, L.M.
Ann. Rev. Biochem., 1992 61 387

Sanderson, S.D., Ember, J.A., Kirnarsky, L., Sherman, S.A., Finch, A.M., Taylor, S.M.
J. Med. Chem., 1994 37 3171-3180

Sanderson, S.D., Kirnarsky, L., Sherman, S.A., Vogen, S.M., Prakesh, O., Ember, J.A., Finch, A.M. and Taylor, S.M.
J. Med. Chem., 1995 38 3669-3675

Siciliano, S.J., Rollins, T.E., DeMartino, J., Konteatis, Z., Malkowitz, L., VanRiper, G., Bondy, S., Rosen, H. and Springer, M.S.
Proc. Nat. Acad. Sci. USA, 1994 91 1214-1218.

Tempero, R.M., Hollingsworth, M.A., Burdick, M.D., Finch, A.M., Taylor S.M., Vogen, S.M., Morgan, E.L., and Sanderson, S.D.
J. Immunol., 1997 158 1377-1382

Stradley, S., Rizo, J., Bruch, M., Stroup, A. and Gierasch, L.
Biopolymers, 1990 29 263-287.

Walker W.R. and Whitehouse, M.W.
Agents & Actions, 1978 8 85

Ward, J.R. and Cloud, R.S.
J. Pharmacol. Exp. Ther., 1966 152 116

Whitehosue, M.W.
Handbook of Animal Models for the Rheumatic Diseases, Eds. Greenwald, R.A., Diamond, H.S., Vol. 1, pp 3-16 CRC Press

Winter, C.A. and Nuss, G.W.
Arth. & Rheumatism, 1966 9 394

Zhang, X., Boyar, W., Galakatos, N. and Gonella, N.C.
Protein Sci., 1997 6 65-72

Zuiderberg, E.R.P., Nettesheim, D.G., Molison, K.W., Carter, G.W.
Biochemistry, 1989 28 172-185; 29 2895-2905.

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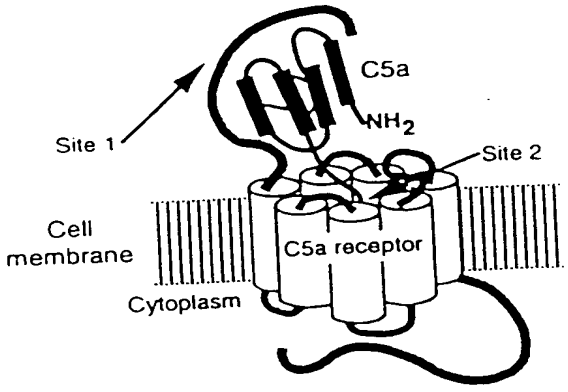
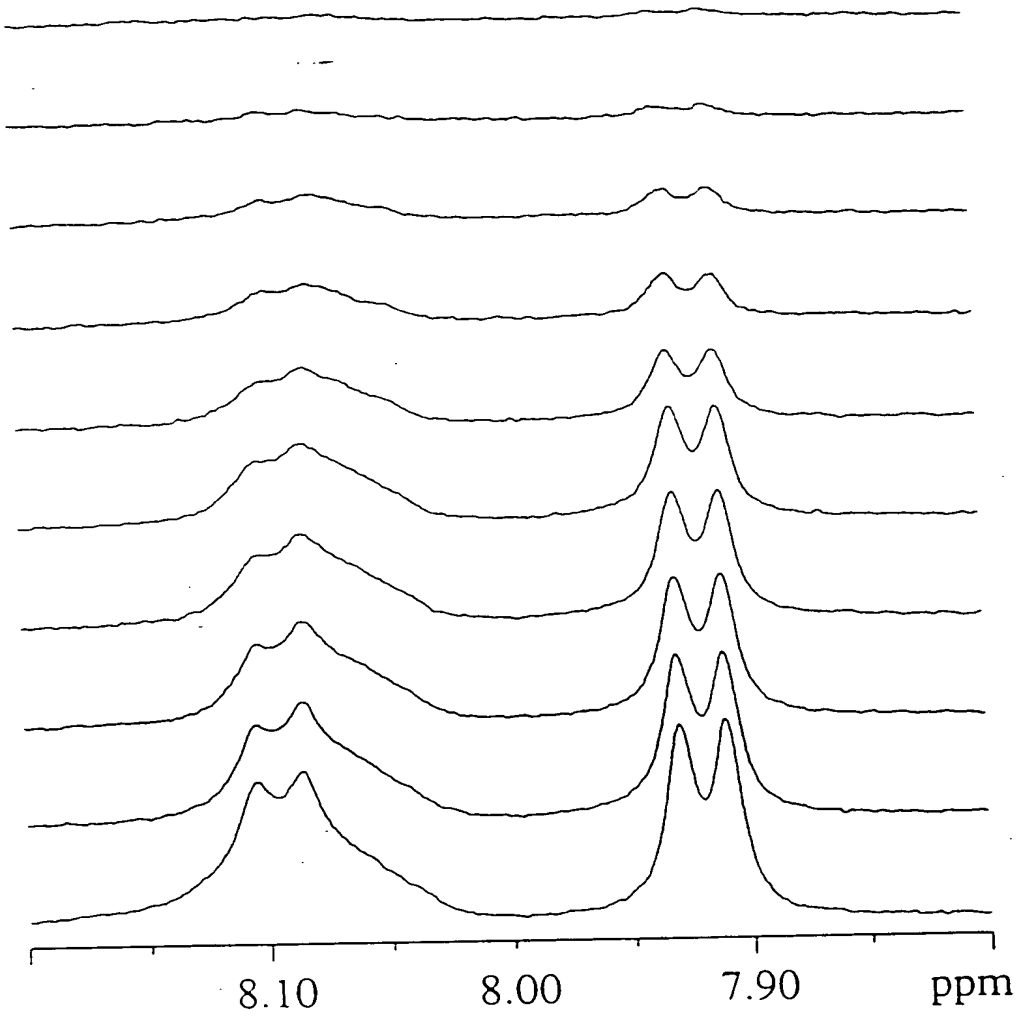


Fig. 1



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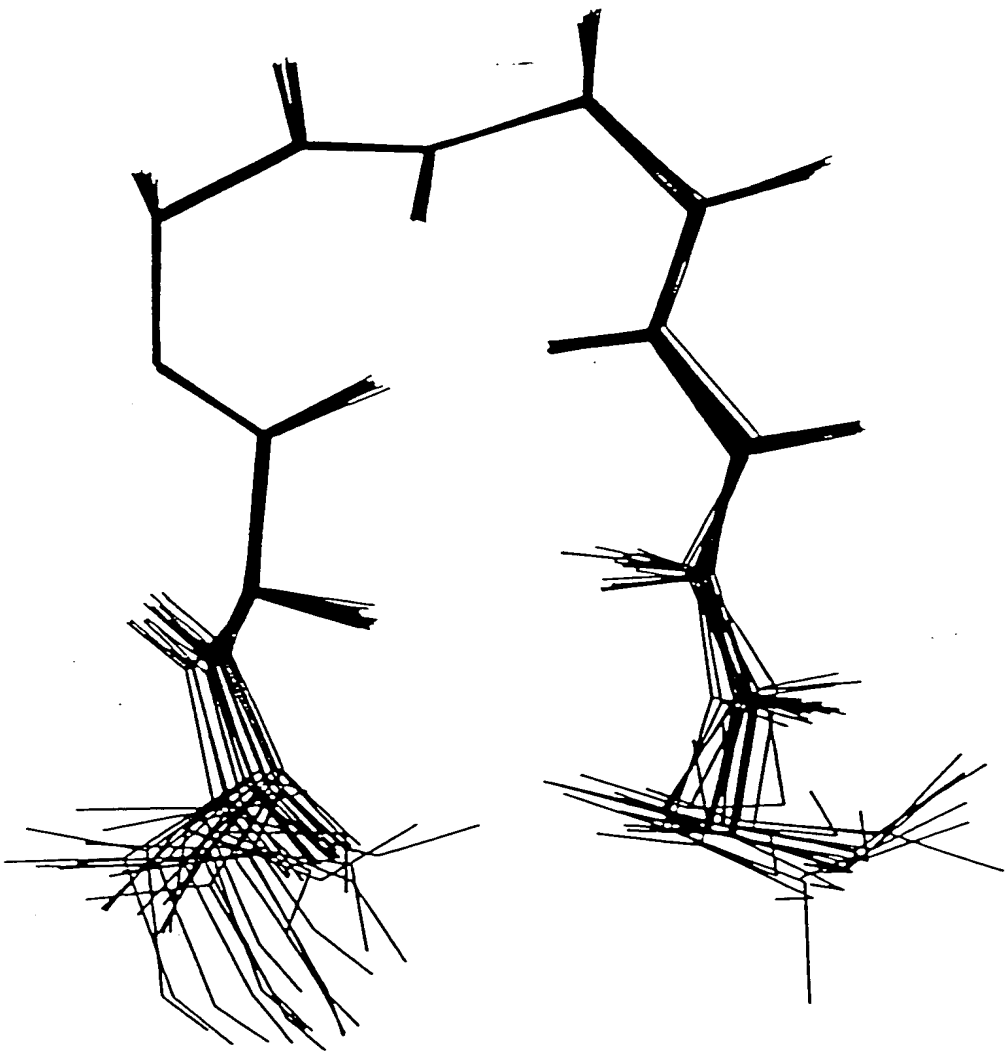

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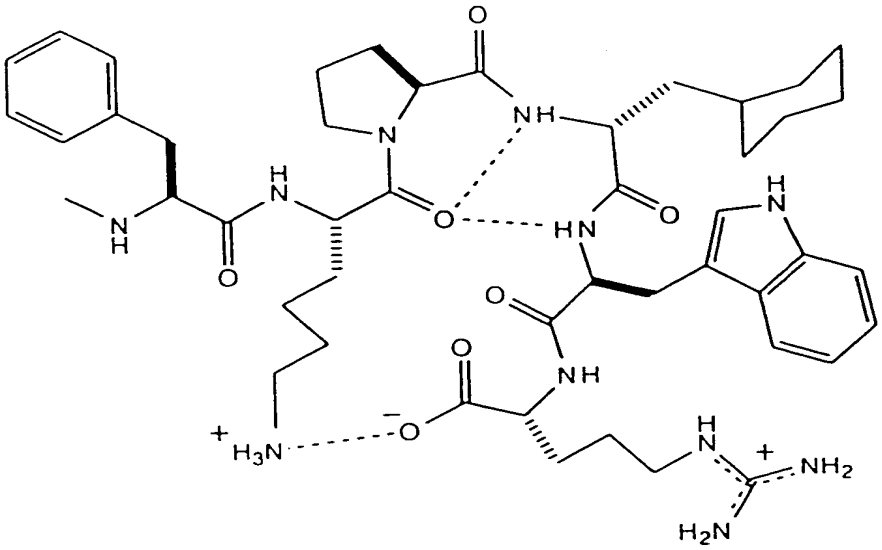

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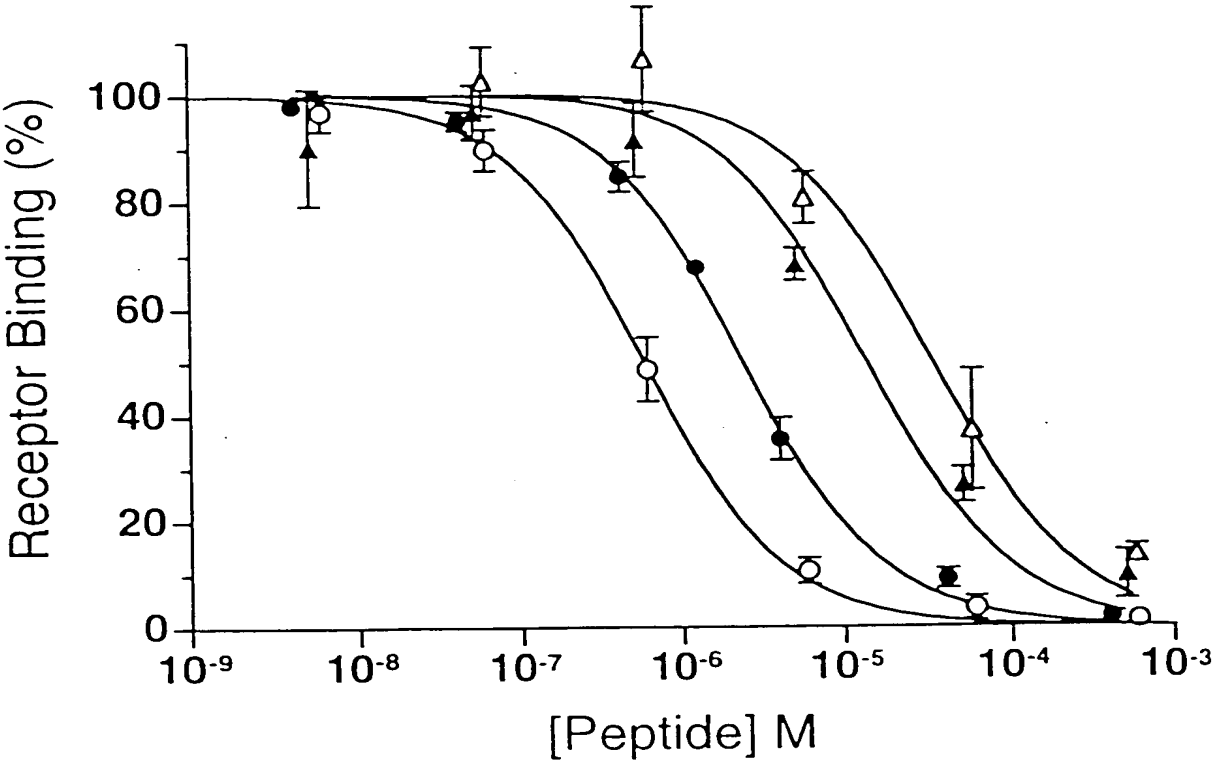
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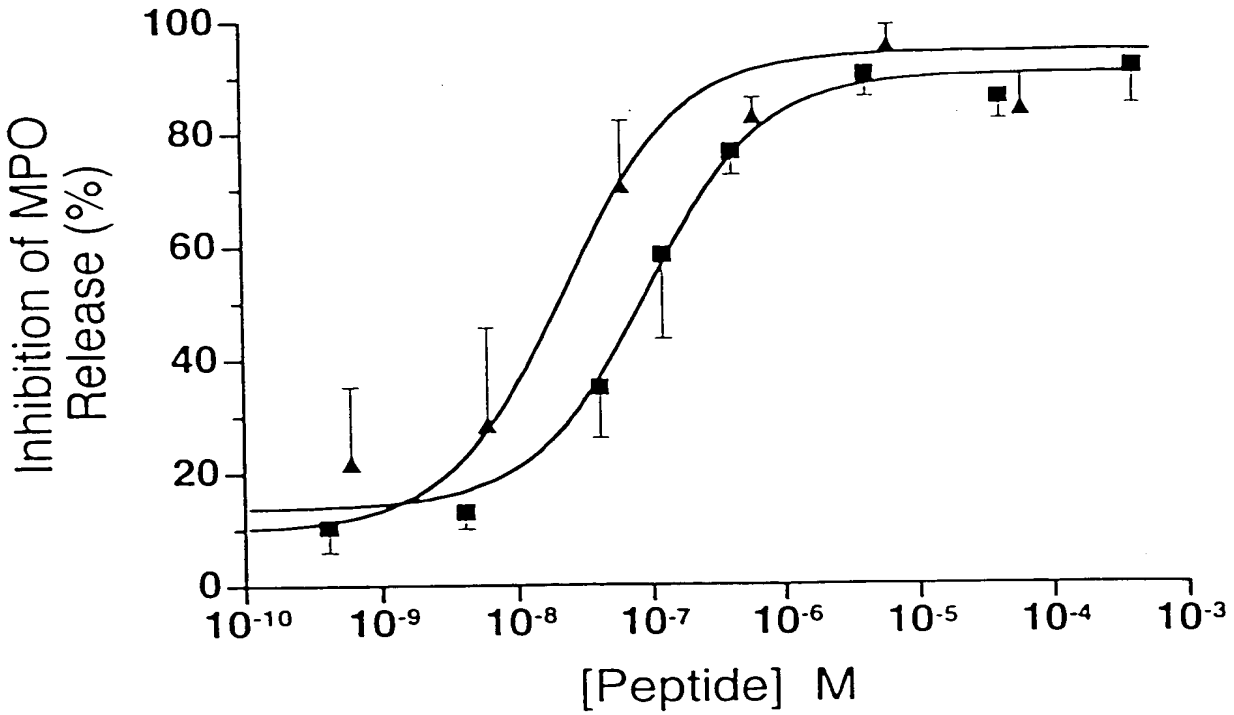
100
80
60
40
20
0
10-9
10-8
10-7
10-6
10-5
10-4
10-3
[Peptide] M

Fig. 5a


Inhibition of MPO Release (%)
100
80
60
40
20
0
10-10
10-9
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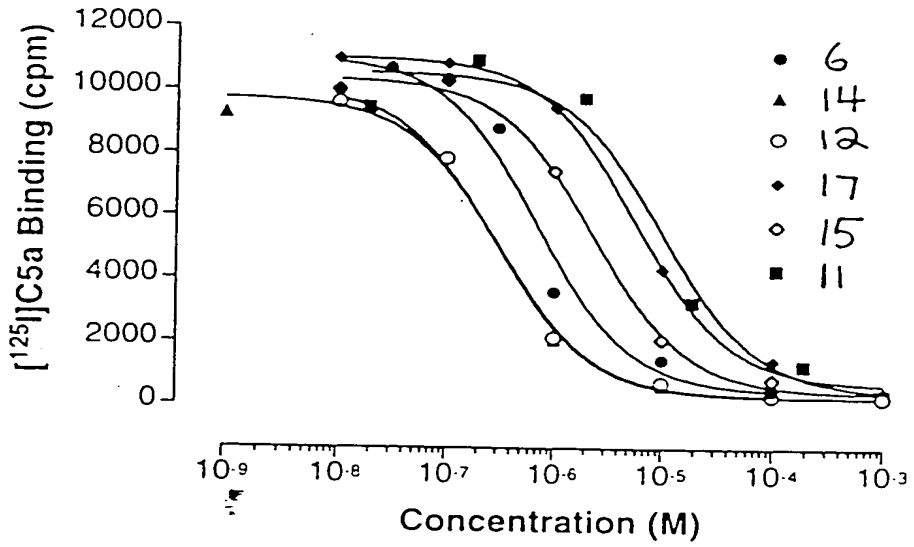
10-7
10-6
10-5
10-4
10-3
[Peptide] M

Fig. 5 b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12000
10000
8000
6000
4000
2000
0
10-9
10-8
10-7
10-6
10-5
10-4
10-3
Concentration (M)
6
14
12
17
15
11

Fig. 6

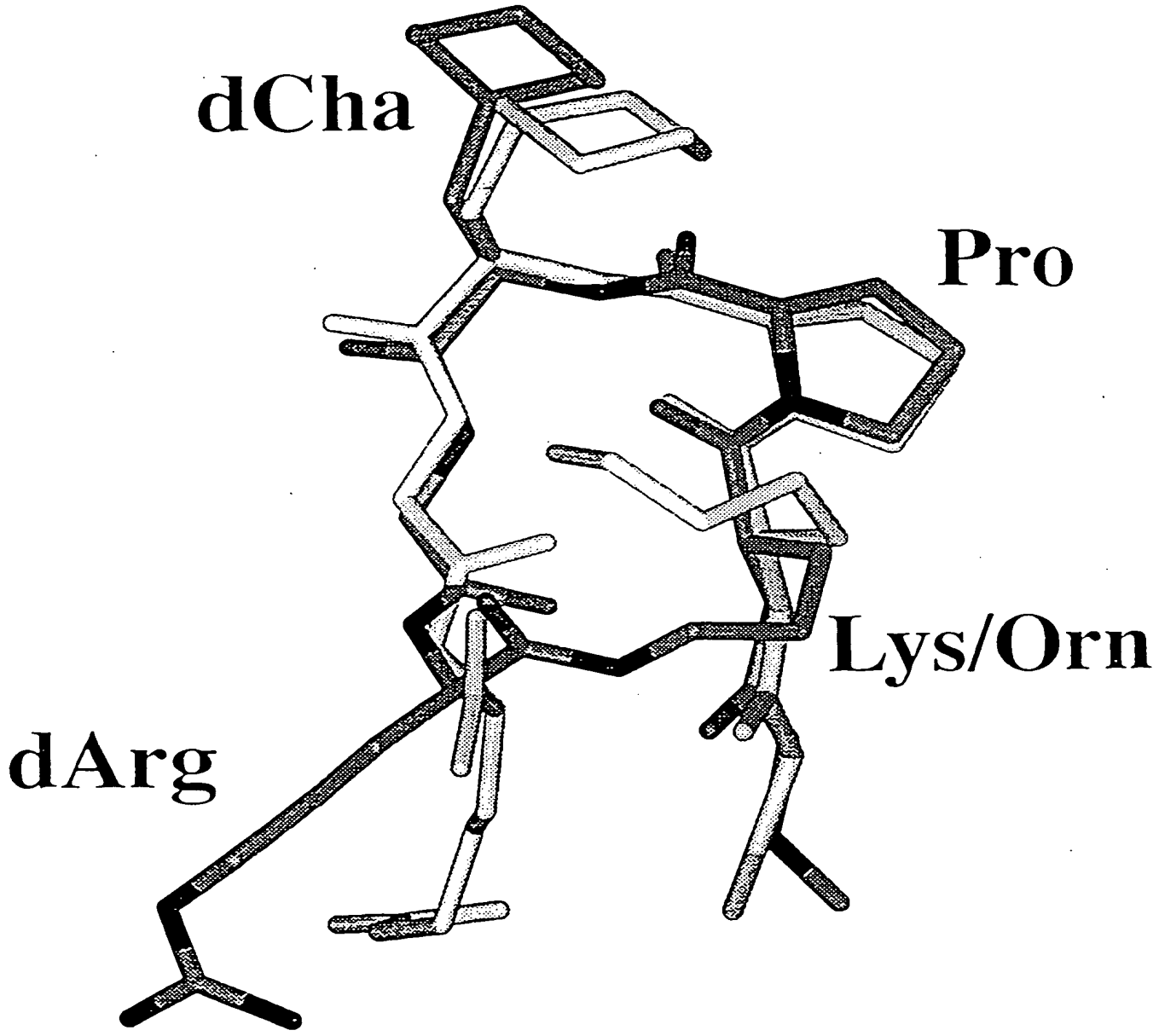

Fig. 7